Quantitative histological analysis of the mandibular branch of the facial nerve in rats[1]

Análise histológica quantitativa do ramo mandibular do nervo facial em ratos

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ABSTRACT

PURPOSE: To establish a model to quantitative histological analysis of the mandibular branch of the facial nerve in rats.

METHODS: Eleven Wistar rats had their right and left mandibular branches of the facial nerve surgically removed and were sacrificed afterwards. Quantitative histological analysis was performed with: a) partial number of axons; b) partial area of the transversal cut of the nerve (9000μm^2); c) partial density. The averages of partial density were obtained. The statistical study was established by Wilcoxon test (p=0.05).

RESULTS: In relation to density of axons, comparison between sides shows no statistically significant difference (p=0.248; p=0.533). Mean partial density of distal and proximal samples was, respectively, 0.18 ± 0.02 and 0.19 ± 0.02 axons/μm^2. Comparison between proximal and distal samples shows no statistically significant difference (p=0.859; p=0.182).

CONCLUSION: This study has successfully established a model to histological quantitative analysis of the mandibular branch of the facial nerve in rats.

Key words: Facial Nerve. Histology. Rats.

RESUMO

OBJETIVO: Estabelecer um modelo para análise histológica quantitativa do ramo mandibular do nervo facial de ratos.

MÉTODOS: Onze ratos Wistar tiveram os ramos mandibulares de seus nervos faciais direito e esquerdo removidos cirurgicamente, e submetidos à análise histológica quantitativa de suas regiões proximal e distal com: a) contagem total do número de axônios, b) medida da área parcial (9000μm^2) de corte transversal do nervo, c) cálculo de densidade parcial (DP).

RESULTADOS: Em relação à densidade dos axônios, a comparação entre os lados não mostrou diferença estatisticamente significativa (p=0,248; p=0,533). A densidade parcial média das amostras distais e proximais foi, respectivamente, 0,18 ± 0,02 e 0,19 ± 0,02 axônios/μm^2. A comparação entre as amostras proximais e distais não mostrou diferença estatisticamente significativa (p=0,859; p=0,182).

CONCLUSÃO: Este estudo estabeleceu com sucesso um modelo de análise histológica quantitativa do ramo mandibular do nervo facial em ratos.

Descritores: Nervo Facial. Histologia. Ratos.

Introduction

Peripheral facial palsy caused by trauma is a very common disease. Permanent search for techniques of repair that improve nerve regeneration stimulated the creation of different types of experimental models with many specimens of animals[1-3].

The mandibular branch of the facial nerve of rats is an excellent material to study diseases caused by trauma and reparation of the facial nerve as it has a long length with no ramification[4], allowing then reparation by using grafts and also giving the possibility to make objective functional study through electromyography. Quantitative histological analysis has been frequently used to quantify regenerated axons and to give an anatomic estimate of nerve regeneration's degree[5-8]. It is directly related to function, because it's known that more number of regenerated myelinated fibers leads to better conduction velocity[9].

There is no description in literature about axons counting of the mandibular branch of the facial nerve in rats, justifying the development of this experimental model.

Methods

The study was approved by the Institutional Ethics Committee for research in animals. The use of laboratory animals follow the Council for International Organization of Medical Sciences ethical code for animal experimentation.

A total of 11 adult male Wistar rats with a mean weight of 250g (ranging from 200 to 300g) were studied.

The rats anesthetized with xylazine hydrochloride (3mg/Kg) and ketamine hydrochloride (80mg/kg) by intraperitoneal injection. Right and left mandibular branches of facial nerve were exposed and removed after fixation. The animals were sacrificed at random (with an intracardiac injection of potassium chloride) for the histological study of the injured facial nerves. The fixation of the facial nerve was carried out *in situ* before sacrificing the animal, by using 2% glutaraldehyde and 1% paraformaldehyde with sodium phosphate buffer (0.1M, pH 7.3). The distal end of the distal and proximal fragments was identified through a diagonal cut and the proximal end was cut transversely for histological analysis. The proximal ends of proximal and distal fragments were situated at, respectively, 28 and 24mm from labial comissure. The specimens were then treated with 2% osmium and dehydrated with ethanol, followed by infiltration with propylene oxide and inclusion with Epoxi® resin (Burlington – Vermont - USA) until polymerization. Transverse 1 µm sections were made and stained with 1% toluidine blue.

Histological observations were carried out using light microscopy (Nikon Eclipse E 600 - Nikon - Japan). The histological slides were photographed with a digital camera (Nikon Coolpix E 955 - Nikon - Japan), recorded in a CD and transferred to a PC for cell count using Sigma Scan Pro 5.0 software (SPSS Science – USA).

A quantitative histological analysis was carried out dividing the number of axons by an area of 9000µm^2 to establish axon density.

Results pertaining to the nerve density were submitted to statistical analysis comparisons using the Wilcoxon test with a significant p value established as less or equal to 0.05.

Results

Figure 1 represents the mandibular branch of the rat facial nerve and Figure 2 illustrates a histological section of a rat facial nerve.

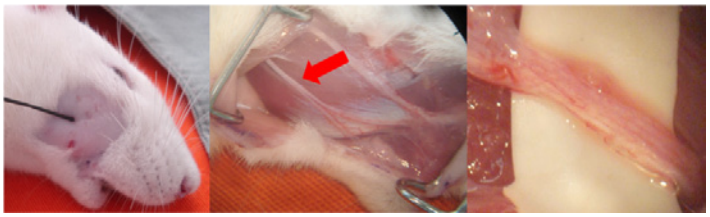

FIGURE 1 - Mandibular branch of the rat facial nerve.

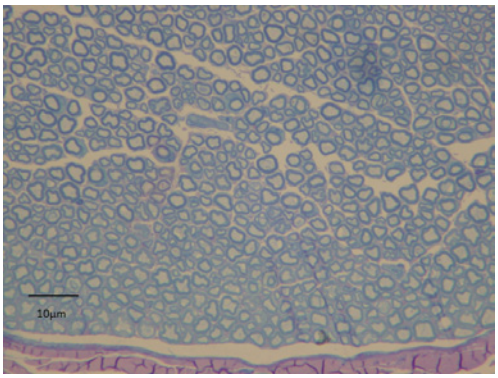

FIGURE 2 - Histological section of a normal rat facial nerve stained with 1% toluidine blue (light microscopy).

On comparing groups regarding density of axons (axons/µm^2), in Table 1, according to Wilcoxon test comparison between sides shows no statistically significant difference between groups (p=0.248; p=0.533). Table 2 describes data without considering sides.

Tables 1, 2 and 3 are related to morphometric neural data of the studied nerves.

TABLE 1 - Density of the myelinated axons compared to the partial area (9000µm²) of the normal mandibular branch of facial nerves of the studied rats. Comparison between sides (Wilcoxon test).

samples	n	Mean	Standand deviation	Inferior limit	Superior limit	Minimum	Maximum	Median	Significance (p)
right proximal	11	0.184	0.015	0.175	0.193	0.148	0.205	0.186	0.248
left proximal	11	0.191	0.017	0.181	0.201	0.165	0.218	0.191	
right distal	11	0.183	0.013	0.175	0.191	0.154	0.199	0.183	0.533
left distal	11	0.185	0.017	0.174	0.195	0.154	0.213	0.184	

TABLE 2 - Density of the myelinated axons compared to the partial area (9000µm²) of the normal mandibular branch of facial nerves of the studied rats.

Nerves	Partial density (axons/µm²)
Distal (n=22)	0.18 ± 0.02
Proximal (n=22)	0.19 ± 0.02

*Mean and standard deviation values.

TABLE 3 - Density of the myelinated axons compared to the partial area (9000µm²) of the normal mandibular branch of facial nerves of the studied rats. Comparison between distal and proximal samples (Wilcoxon test).

samples	n	Mean	Standand deviation	Inferior limit	Superior limit	Minimum	Maximum	Median	Significance (p)
right proximal	11	0.184	0.015	0.175	0.193	0.148	0.205	0.186	0.859
right distal	11	0.183	0.013	0.175	0.191	0.154	0.199	0.183	
left proximal	11	0.191	0.017	0.181	0.201	0.165	0.218	0.191	0.182
left distal	11	0.185	0.017	0.174	0.195	0.154	0.213	0.184	

In Table 3, that compares proximal and distal samples, there was no statistically significant difference between groups (p=0.859; p=0.182) by Wilcoxon test.

Discussion

Quantitative study of the mandibular branch of the facial nerve in rats was performed by the analysis of the density of axons by mm², comparing densities of number of axons in a defined partial area. In agreement with most authors, this study opted for the analysis of density (number of myelinated axons) in relation to neural area and not axonal area[9,10].

Sampling methods have been used to count neural fibers[5-12] the samples of this work corresponded to almost 90% of the total area of the nerves, which was more than sufficient to obtain accuracy of the method[12].

Left and right sides were compared in these normal nerves, and as it wasn't any statistical difference, they were considered as same side. Therefore, casuistic was doubled to obtain a histological pattern of mandibular branch of the facial nerve in rats.

As it was mentioned above, there are no papers in literature describing number of axons of rat's facial nerve mandibular branch to liken to this one.

Distal and proximal analyses were made to correlate the number of axons in different segments of the same nerve. It will make possible the comparison with injured nerves submitted to different types of surgical repair.

Conclusion

This study has successfully established a model for histological quantitative analysis of the mandibular branch of the facial nerve in rats.

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